6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, and/or measuring, and/or monitoring 1,4-dichlorobenzene, its metabolites, and other biomarkers of exposure and effect to 1,4-dichlorobenzene. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

6.1 BIOLOGICAL SAMPLES

Methods are available for measuring levels of 1,4-dichlorobenzene in blood, urine, tissue, and breath. Representative methods are summarized in Table 6-1. Methods include sample collection, preparation and cleanup and determination. Sample preparation techniques are usually required to separate the compound of interest from the complex biological sample medium. Gas purge and solvent extraction are used most frequently to separate 1,4-dichlorobenzene from blood, urine, and tissues. The breath matrix is relatively simple and does not require preparation steps; however, special techniques such as use of a spirometer are required to provide pure air for inhalation and a mechanism for collection of exhaled air. Gas chromatography (GC) is used most frequently to detect 1,4-dichlorobenzene in biological materials. Detectors used to identify 1,4-dichlorobenzene in biological materials include the electron capture detector (ECD) (Bristol et al. 1982; Jan 1983) the photoionization detector (PID) (Langhorst and Nestrick 1979), and mass spectrometry (MS) (Ashley et al. 1992; Michael et al. 1980). ECD and PID provide some selectivity, but confirmation using a different GC column or detector is often recommended. MS provides identification as well as quantitation of analytes.

Separation of 1,4-dichlorobenzene from biological samples may be accomplished by extraction with hexane (Bristol et al. 1982; Jan 1983), or carbon tetrachloride (Langhorst and Nestrick 1979), or by purging with an inert gas and trapping on a sorbent material. Solvent extraction permits concentration, thereby

Table 6-1. Analytical Methods for Determining 1,4-Dichlorobenzene in Biological Materials

Sample matrix	Sample preparation	Analytical method	Sample detection limit	Percent recovery	Reference
Blood	Headspace purge; thermal desorption	cap. GC/MS	≈3 ng/mL	86.3[a]	IARC Method 25; Pellizzari et al. 1985
Blood	Headspace purge; thermal desorption	cap. GC/MS	Low-ppb level	86–120 (model compounds)	Michael et al. 1980
Blood	Solvent extraction; silica gel column clean-up	GC/PID	3 ppb	89	Langhorst and Nestrick 1979
Blood	Solvent extraction	GC/ECD	2 ppb	81.6	Bristol et al. 1982
Blood	Purge and trap	cap. GC/MS	0.04 ppb	93–98	Ashley et al. 1992
Blood, urine	purge-and-trap, thermal desorption cap	GC/MS	No data	No data	Barkley et al. 1980
Urine	Solvent extraction; silica gen column clean-up	GCPID	0.75 ppb	81	Langhorst and Nestrick 1979
Urine	Headspace purge; thermal desorption	cap. GC/MS	Low-ppb level	48–110 (model compounds)	Michael et al. 1980
Adipose tissue	Maceration; headspace purge; thermal desorption	cap. GC/MS	Low-ppb level	13–80 (model compounds	Michael et al. 1980
Human milk	Headspace purge; thermal desorption	GC/MS	0.6	62.9[b]	Erickson et al. 1980
Human milk	Solvent extraction; cleanup with sulfuric acid, Florisil	GC/ECD	5 ppb	>80	Jan 1983
Adipose tissue	Solvent extraction; cleanup with sulfuric acid, Florisil	GC/ECD	146	>80	Jan 1983
Tissue	Maceration; headspace purge; thermal desorption	cap. GC/MS	6 ng/g	No data	IARC Method 25; Pellizzari et al. 1985

Table 6-1. Analytical Methods for Determining 1,4-Dichlorobenzene in Biological Materials (continued)

Sample matrix	Sample preparation	Analytical method	Sample detection limit	Percent recovery	Reference
Breath	Collection using a spirometer; adsorption on Tenax traps; thermal desorption cap	GC/MS	No data	No data	Barkley et al. 1980
Breath	Collection into canisters using spirometer; cryofocussing; thermal desorption	cap. GC/MS-SIM	low-µg/m^3 levels	49–80	Thomas et al. 1991

[a] Value is for m-dichlorobenzene
[b] Value is for chlorobenzene

cap. = capillary; ECD = electron capture device; GC = gas chromatography; MS = mass spectrometry; SIM = selected ion monitoring

increasing sensitivity, but the extraction solvents can interfere with the analysis, and evaporative losses can result in low recovery. Gas purge techniques may be static (headspace) or dynamic (purge-and-trap). The static headspace technique is relatively simple, but may be less sensitive than the purge-and-trap method. The purge-and-trap method, while providing increased sensitivity, requires more complex instrumentation and may result in artifact formation (Seto 1994).

Although a variety of methods are available for determination of 1,4-dichlorobenzene in blood, few are well characterized and validated. A method has been developed which utilizes headspace purge followed by thermal desorption of the trapped, purged analytes. 1,4-Dichlorobenzene is then determined by capillary GC/MS (Michael et al. 1980; Pellizzari et al. 1985). Recovery is very good (>85%) and detection limits are in the low-ppb range for model compounds (Michael et al. 1980; Pellizzari et al. 1985). Performance data are not available for 1,4-dichlorobenzene. A sensitive and reliable method for identification and quantitation of 1,4-dichlorobenzene in samples of whole blood has been developed by Ashley and coworkers at the Centers for Disease Control and Prevention (CDC) (Ashley et al. 1992). The method involves purge-and-trap of a 10 rnL blood sample with analysis by capillary CC/high resolution MS. Antifoam procedures are utilized as well as special efforts to remove background levels of volatile organic compounds (VOCs) from reagents and equipment. The method is sensitive enough (ppt levels) to determine background levels of VOCs in the population and provides adequate accuracy (93-98% recovery) and precision (21% RSD) for monitoring 1,4-dichlorobenzene in the general population.

Methods are available for monitoring 1,4-dichlorobenzene in urine and tissues, particularly adipose tissue and mother’s milk. Solvent extraction, silica gel column clean-up, and GC/ECD or GC/PID analysis has been used for urine (Langhorst and Nestrick 1979), mother’s milk (Jan 1983), and adipose tissue (Jan 1983). Recovery is good (>80% recovery) and detection limits are in the low-ppb range (Jan 1983; Langhorst and Nestrick 1979). Headspace purge, followed by capillarity GC/MS analysis has been utilized for urine (Michael et al. 1980), mother’s milk (Erickson et al. 1980) and tissue (Pellizzari et al. 1985). Recovery, where reported, is adequate (>60%) (Erickson et al. 1980), and detection limits are in the low-ppb range (Erickson et al. 1980).

Breath samples are usually collected through a spirometer onto a sorbent cartridge (Barkley et al. 1980) or into passivated canisters (Thomas et al. 1991). Analytes are concentrated cryogenically from a portion of the canister contents or after thermal desorption from the sorbent, then analyzed by GC/MS. Recovery

using Tenax cartridges is 87-101%, precision for side-by-side samples is 530% RSD, and the detection limit is ≈1 μg/m^3 (Wallace 1987). The method is sufficiently sensitive and reliable for monitoring exposure to 1,4-dichlorobenzene. Recovery for collection in canisters is 49-80%, precision is <20% and the detection limits are in the low-μg/m^3 range (Thomas et al. 1991). The spirometer system utilizing canisters is compact, and may be useful as a field screening method (Thomas et al. 1991).

6.2 ENVIRONMENTAL SAMPLES

Methods are available for determining 1,4-dichlorobenzene in a variety of environmental matrices. A summary of representative methods is shown in Table 6-2. Validated methods, approved by agencies and organizations such as EPA, ASTM, APHA, and NIOSH, are available for air, water, and solid waste matrices. These methods for analysis of drinking water, waste water, and soil/sediment samples are included in Table 6-2. Many of the methods published by APHA (1995) and ASTM (1994) for water are equivalent to the EPA methods.

GC is the most widely used analytical technique for quantifying concentrations of 1,4-dichlorobenzene in environmental matrices. Various detection devices used for GC include the flame ionization detector (FID), ECD, Hall electroconductivity detector (HECD), and PID. Confirmation using a second column is usually recommended. MS provides identification as well quantitation for GC analysis. Because of the complexity of the sample matrix and the usually low concentrations of VOCs in environmental media, sample concentration is generally required prior to GC analysis. Methods suitable for determining trace amounts of 1,4-dichlorobenzene in aqueous and other environmental media include three basic approaches to the pretreatment of the sample: gas purge-and-trap technique, headspace-gas extraction, and extraction with solvent. Care must be taken during sample collection and processing to avoid evaporative losses. Contamination is another potential analytical problem and monitoring is required. 1,4-Dichlorobenzene is a relatively common chemical compound and can contamination reagents and glassware.

Charcoal adsorbent is used for collection of 1,4-dichlorobenzene in occupational air. The compounds are desorbed with carbon disulfide and analyzed by GC/FID. The method is sufficiently sensitive and reliable for determining occupational exposure to 1,4-dichlorobenzene (NIOSH 1994).

Table 6-2. Analytical Methods for Determining 1,4-Dichlorobenzene in Environmental Samples

Sample matrix	Sample preparation	Analytical method	Sample detection	Percent recovery	Reference
Occupational air	Collection on charcoal tubes; desorption with CS_2	GC/FID	0.01 mg/ sample[a]	± 12.5	Method 1003 NIOSH 1994
Ambient air	Collection in canisters; cryofocussing; thermal desorption	cap. GC with FID, ECD or MS	No data	No data	Method TO-14 EPA 1988a
Air–emissions sources	MM5 sampling train (condensate, filter, adsorbent); condensate, impinger and rinses, solvent extraction, evaporation; XAD-2 adsorbent and filters, Soxhlet extraction, concentration	cap. GC/MS	No data	Bias -13 to -16 for selected compounds	Method 0010 EPA 1994f
Air–emission sources	VOST sampling train (sorbent traps); thermal desorption	GC/MS	No data	No data	Method 0030 EPA 1994h
Drinking water	Purge and trap	GC/HECD; conf. on second col. or GC/MS	<0.01 µg/L for most VOCs	90	Method 502.1 EPA 1991a
Drinking water	Purge and trap	GC/PID-HECD; conf. by GC/MS	0.01–0.03 µg/L (PID); 0.01–0.04 µg/L (HECD)	97–103 (PID); 97–98 (HECD)	Method 502.2 EPA 1991b
Drinking water	Purge and trap	GC/PID; conf. on second col. or GC/MS	0.006 µg/L	91–107	Method 503.1 EPA 1991c
Drinking water	Purge and trap	cap. GC/MS	0.03–0.04 µg/L	93–103	Method 524.2 EPA 1992a

Table 6-2. Analytical Methods for Determining 1,4-Dichlorobenzene in Environmental Samples (continued)

Sample matrix	Sample preparation	Analytical method	Sample detection	Percent recovery	Reference
Waste water	Purge and trap	GC/HECD; conf. on second col. or GC/MS	0.24 µg/L	97.5	Method 601 EPA 1984c
Waste water	Purge and trap	GC/PID; conf. on second col. or GC/MS	0.3 µg/L	120	Method 602 EPA 1984f
Waste water	Solvent extraction; optional Florisil column clean-up	GC/ECD	1.34 µg/L	89	Method 612 EPA 1984c
Waste water	Purge and trap	GC/MS	No data	No data	Method 624 EPA 1984d
Waste water	Purge and trap	GC/MS	Not reported	Not reported	Method 6210 B APHA 1995a
Waste water	Purge and trap	GC/MS	0.1–0.5 µg/L (all VOCs)	105	Method 6210 C APHA 1995b
Waste water	Purge and trap	cap. GC/MS	0.02–0.2 µg/L (all VOCs)	103–106	Method 6210 D APHA 1995c
Waste water	Purge and trap	GC/PID; conf. on second col.	0.3 µg/L		Method 6220 B APHA 1995d
Waste water	Purge and trap	GC/PID	0.01–0.05 µg/L (all VOCs)	91–107	Method 6220 C APHA 1995e
Waste water	Purge and trap	GC/HECD; conf. on second col.	0.24 µg/L		Method 6230 B APHA 1995f
Drinking water	Purge and trap	GC/HECD (optional PID); conf. on second col.	0.01–0.05 µg/L	90	Method 6230 C APHA 1995g

Table 6-2. Analytical Methods for Determining 1,4-Dichlorobenzene in Environmental Samples (continued)

Sample matrix	Sample preparation	Analytical method	Sample detection	Percent recovery	Reference
Drinking water	Purge and trap	cap. GC/PID, HECD	Not reported	103 (PID); 98 (HECD)	Method 6230 D APHA 1995h
Drinking water	Purge and trap	GC	low µg/L	99 (all VOCs)	Method D 3871 ASTM 1994
Solid waste	Purge and trap or direct injection	GC/HECD; conf. on second col.	Not reported	≈90	Method 5030A EPA 1994a; Method 8010B EPA 1994b
Solid waste	Purge and trap or direct injection	GC/PID; conf. on second col.	3–250 ppb (purge and trap)	≈90	Method 8020A EPA 1994c
Solid waste	Purge and trap or direct injection	cap. GC/HECD, PID	0.1–5 µg/L (HECD); 0.07–3.5 (PID)	91 (HECD); 103 (PID)	Method 8021A EPA 1994d
Solid waste	Various injection options	GC/ECD	13.4–900 µg/L	Not reported	Method 8120A EPA 1994e
Solid waste	Purge and trap	cap. GC/MS	1–15 µg/L	103–106	Method 8260A EPA 1994g

cap. = capillary; conf. = confirmation; col. = column; ECD = electron capture detector; FID = flame ionization detector; GC = gas chromatography; HECD = Hall electrolytic conductivity detector; MS = mass spectrometry; PED= photoionization detector; VOC = volatile organic compound

Ambient air samples are collected on adsorbents such as Tenax (Wallace 1987), or multisorbent (Heavner et al. 1992; Oliver et al. 1996) or in passivated canisters (EPA 1988a). Tenax traps are thermally desorbed, concentrated cryogenically, and analyzed by capillary GC/MS (Wallace et al. 1987). Recovery is good (81-110%), precision for side-by-side samples is acceptable (9-45% RSD), and the detection limit is ≈ 1 μg/m^3 (Wallace 1987). Multisorbent traps may be solvent desorbed and analyzed by capillary GC/MS. Recovery and precision are good and detection limits as low as 0.019 ppb have been reported (Oliver et al. 1996). Collection of air samples in passivated stainless steel canisters is also widely utilized (EPA 1988a), but performance data are unavailable. Passive sampling devices are also widely used, due in part to their ease of use and small size (Lewis et al. 1985).

For water, soil, or sediment samples, 1,4-dichlorobenzene is purged from the sample with an inert gas such as helium or nitrogen, and then passed through the sorbent (EPA 1984a, 1984b, 1991a, 1991b, 1991c, 1992a, 1994a, 1994f). The analytes are thermally desorbed and analyzed by GC/HECD, GC/PID, GUECD, or GC/MS techniques. Detection limits for waste waters and solid wastes are in the low-ppb range, which is probably well below levels of health concern. Detection limits for drinking water samples are in the ppt range (0.006-0.04 μg/L) (EPA 1991a, 1991b, 1991c, 1992a).

Several physical parameters may interfere with analytical accuracy. High sampling flow rates and high temperature and humidity may cause decreased adsorption of 1,4-dichlorobenzene vapor on the solid sorbent (APHA 1995a). Interference by other VOCs with similar retention times may be resolved by using different GC column materials and temperatures or be using MS techniques.

The use of capillary columns rather than packed column GC has improved resolution and sensitivity and shortened the analysis time (Washall and Wampler 1988). However, more stringent sample clean-up procedures are required for capillary column GC (Oliver and Nicol 1982b). The development of methods using whole column cryotrapping (Pankow and Rosen 1988; Pankow et al. 1988) and cryogenic refocusing (Washall and Wampler 1988) provide even greater sensitivity and resolution for GC analysis.

6.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate

information on the health effects of 1,4-dichlorobenzene is available. Where adequate information is not available, ATSDR, in conjunction with the NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of 1,4-dichlorobenzene.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. Exposure to 1,4-dichlorobenzene may be evaluated by measuring the levels of this compound in blood, breath, milk, and adipose tissue, and by measuring the level of 2,5-dichlorophenol, a metabolite of 1,4-dichlorobenzene, in urine (Bristol et al. 1982; Erickson et al. 1980; Jan 1983; Langhorst and Nestrick 1979; Pellizzari et al. 1985). Sensitive analytical methods are available for measurements in blood. Development of methods with improved specificity and sensitivity for other tissues and breath would be valuable in identifying individuals with low-level exposure. Development of standardized procedures would permit comparison of data and facilitate the study of correlations between exposure and measured levels biological samples. Interlaboratory studies are also needed to provide better performance data for methods currently in use.

There are no known health effects such as elevated liver enzymes that are uniquely associated with exposure to 1,4-dichlorobenzene. Therefore, the identification of specific health effects and the development of analytical methods to determine biomarkers of effect for 1,4-dichlorobenzene would be useful.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Air is the environmental medium of most concern for human exposure to 1,4-dichlorobenzene. Exposure from drinking water may also be of concern in some areas, such as near hazardous waste sites. Existing analytical methods can measure 1,4-dichlorobenzene in these and other environmental media at

background levels (EPA 1988a, 1984a, 1984b, 1991a, 1991b, 1991c, 1992a, 1994a, 1994f; NIOSH 1994). The accuracy and precision of the methods for water and wastes are well documented and MS provides adequate specificity. Performance data for measurements in ambient and indoor air would be helpful. Development of techniques to improve the accuracy and ease of sample preparation and transfer for these methods would also be helpful.

6.3.2 Ongoing Studies

The Environmental Health Laboratory Sciences Division of the National Center for Environmental Health, Centers for Disease Control and Prevention, is developing methods for the analysis of 1,4-dichlorobenzene and other volatile organic compounds in blood. These methods use purge and trap methodology, high resolution gas chromatography, and magnetic sector mass spectrometry which gives detection limits in the low parts per trillion (ppt) range.

The U.S. EPA is conducting a pilot program for comprehensive monitoring of human exposure. The National Human Exposure Assessment Study (NHEXAS) is being conducted in three regions of the United States. in order to establish relationships between environmental concentrations, exposure, dose and health response and to determine the incidence and causes of high exposures, especially for biologically susceptible persons. One of the aims of the pilot study is to test measurement methodology for a variety of pollutants, including 1,4-dichlorobenzene in air and water. As an adjunct to this pilot study, the U.S. EPA and the State of Minnesota are conducting a study of children’s exposure to toxic chemicals, including 1,4-dichlorobenzene.